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(54) **Multi-cultured yogurt, solid spread and cottage cheese.**

(57) This invention is for a multi-cultured yogurt or dairy spread such as cottage cheese and the process for making them. Yogurt is normally produced by inoculating milk with L. bulgaricus and S. thermophilus then letting fermentation break down the milk lactose. The accomplishment of this invention is the recognition that more lactose can be broken down by adding a second fermentation step to the usual yogurt making process. The result is that one maximizes the breakdown of milk lactose into glucose and galactose while enhancing the amount of enzyme lactase in the finished product. B. bifidum or L. acidophilus are disclosed as the preferred microorganisms used in the second fermentation steps.

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MULTI-CULTURED YOGURT, SOLID SPREAD  
AND COTTAGE CHEESE

This invention relates to fermented or cultured dairy products and the process for their manufacture wherein various microorganisms are used to alter the composition of milk to make a more palatable and healthful product. Particularly, the area of invention is  
5 directed to the art of making yogurt or a solid spreadable food product or cottage cheese by employing different microorganisms in sequential fermentation stages. More particularly, the invention relates to various methods for preparing dairy products containing Bibidobacterium  
10 bifidum.

Man's knowledge of yogurt making goes back to ancient times; as a consequence, little is known concerning the actual origin of yogurt. It is known, however, that yogurt was first used in the Middle East or  
15 Asia-Minor. Supposedly, yogurt was discovered by nomadic herdsmen who carried milk in a vessel made from a sheep's stomach. It was later found to be that the sheep's stomach was the natural habitat of the yogurt-making microorganisms. The domain of the herdsmen was the desert  
20 and consequently the milk contents was exposed to alternate rapid heating and rapid cooling. During the heat of the day, the microorganisms would multiply rapidly and convert the disaccharide sugar or lactose into glucose and galactose. In the cool of the night, the microbial  
25 action would be brought to a halt leaving a custard-like substance. The early herdsmen learned that this yogurt was more digestible than the milk from which it originated. Later it was discovered that the yogurt making process could be carried out in earthenware vessels

if a small amount of yogurt was used to begin the process anew.

5       The yogurt of ancient times was very much  
different from what is now known as yogurt. It was not  
until the yogurt-making microorganisms were identified  
that pure yogurt could be manufactured. United States  
Patent No. 1,710,133 to Winkler discloses that the use of  
10   pure Lactobacillus bulgaricus to culture milk provides a  
far more palatable food product and avoids the problems  
associated with using impure substances. Winkler also  
teaches the importance of single fermentation in the  
production of yogurt.

15       Preparation of the microorganism cultures used to  
ferment milk is discussed in United States Patent No.  
3,480,443 to Schuler. The Schuler process also emphasizes  
the method of single culturing.

20       The advantage of yogurt or fermented dairy  
products over milk is that the microorganisms convert  
substantial quantities of lactose, or milk sugar, into  
glucose. Lactose is not readily digested by most humans  
and must first be converted to glucose and galactose,  
25   usually in the stomach. Although the yogurt making  
process will achieve some lactose conversion much lactose  
still remains present. It was to this problem that my  
prior invention, disclosed in United States Patent No.  
4,034,115, was directed.

30       According to my prior invention, skim milk,  
ordinary milk, with or without non-fat milk solids, is  
raised to a temperature suitable for the culturing process  
and inoculated with lactose-forming bacteria cultures used  
35   in the production of ordinary buttermilk. Fermentation is

allowed to continue until the lactose is hydrolyzed as fully as possible into glucose and galactose. At the point where approximately 80 percent of the lactose remains the mixture is then inoculated a second time with  
5 yogurt-making bacteria and allowed to ferment. The second fermentation further reduces the lactose and is allowed to continue until the curd starts to separate from the whey. The substance is then allowed to cool and thereupon small amounts of colostrum are added. The mixture is then  
10 inoculated a third time and allowed to ferment completing the transformation of the lactose as fully as possible.

My prior invention, though offering substantial improvements over the prior art, had some disadvantages  
15 with regard to the production of a commercially practicable product. My prior invention required three or more fermentations whereas the method of the present invention requires only two. Moreover, the restricted availability of colostrum places severe limitations on the  
20 quantity of product that can be produced by the method of my prior invention. Not only does my present invention result in a simpler method of manufacture, it eliminates the need for colostrum and the third fermentation step, but it also has the advantage of producing a more  
25 flavorful product than was achieved by my prior invention. Not only does my present invention remove lactose in substantial quantities, but accomplishes that goal by improving the nutritional content of the substance.

30

This invention relates to a multi-cultured milk product with an acceptable and palatable flavor and the processes for their preparation. More specifically, this  
35 invention relates to a unique multi-cultured milk product

and a process that reduces the disaccharide sugar or lactose more completely than heretofore thought possible thereby increasing the amount of glucose and galactose. Prior milk products that have been prepared by multiple  
5 fermentation have used either alcohol or heat during the preparation which have the effect of hindering the enzymatic transformation of lactose to glucose and galactose. The use of alcohol in a milk product through some form of fermentation with yeast inhibits some of the  
10 enzymes normally present in milk and produces food of lessened nutritional content. Moreover, some people are allergic to the lactose in milk itself and are, therefore, denied the benefits of a milk product. For millions of people, intolerance develops during the life cycle such  
15 that they are unable to easily digest lactose, a condition that is believed to arise from the deficiency of B-galactosidase in the intestinal microville.

Previously, bacteria used as starters for  
20 culturing various types of milk-based products have included spherical types such as Streptococcus lactis, Streptococcus cremoris, Leuconostoc citrovorum, Streptococcus diacelelaticus, and Streptococcus durans which are used primarily for buttermilk and sour cream  
25 while rod-shaped types such as Lactobacillus bulgaricus, Lactobacillus heleveticus, Lactobacillus lactis, Lactobacillus acidophilus, Actinomyces bifidus, and Doderlein's bacillus are used primarily for yogurt-like products. However, such milk-based products generally  
30 have a lactose content after one culturing still higher than it need be.

In previously developed processes, the lactose was broken down by various fermentation processes  
35 converting the lactose into lactic acid as when milk with or without non-fat milk solids, partly skim milk or skim

milk is changed into yogurt or buttermilk; however, over 80 percent of the disaccharide sugar may remain unspent after the buttermilk and sour cream fermentation is completed. Further efforts to remove the lactose have  
5 resulted in the past in lowering the nutritional value of the milk product or producing products that are unpalatable.

It has also been incorrectly assumed that  
10 cultures must be balanced when used together. One culture will reach a peak at which curd starts to separate from the whey. At this point, the addition of different varieties of souring cultures will produce new curdling as though no souring had occurred when the product is brought  
15 to a temperature suitable for optimal growth. Here, temperature is the controlling factor.

An inexpensive process to produce milk products digestible to the multitude of people who cannot drink it  
20 would have far-reaching effects, particularly if substantial quantities of lactose can be transformed without altering the nutritional value of the milk products, except to improve it.

25 The therapeutic value of inoculating milk with various strains of microorganisms has been reported by numerous authorities to be particularly important for maintaining good intestinal metabolism and health. Three species of such microorganism cultures include  
30 Bifidobacterium bifidum, L. acidophilus, and L. bulgaricus. L. bulgaricus is used in the production of

yogurt and is believed to prolong human life by inhibiting the growth of proteolytic microbes in the intestinal tract; however, the culture will not survive in the digestive tract for prolonged periods of time. L. acidophilus and B. bifidum, on the other hand, are known to be able to retain their viability through the digestive tract, displacing proteolytic bacteria completely, thus affording a means of implantation in the lower intestinal tract. Both cultures have the capabilities of maintaining a normal microbiological balance, particularly in the intestinal flora, suppressing many undesirable organisms while promoting a beneficial metabolism. L. acidophilus is completely non-pathogenic and offers an entirely safe, therapeutic regimen.

Until a few years ago, the beneficial effects resulting from the ingestion of L. acidophilus in cases of gastrointestinal disturbances were attributed to the simple outgrowth of the offending pathogen by the lactobacilli. Recently, a number of workers established that there is more to the reaction than just competition between strains. A by-product produced by L. acidophilus has been called variously antagonist (White and Hall, J. Dental Res. 48272, 1949); lactobacillin (Vincent et al., ATE Nature 168, 659, 1951); lactocidin (Vincent et al., J. Bact. 78, 479, 484, 1959)); and antibiotic (Shahani et al., U.S. Pat. No. 3,689,640).

The form in which L. acidophilus has been usually employed to obtain its therapeutic effect has been as fermented milk. The number of viable organisms in acidophilus milk was usually in the order of 250 million per milliliter which produced a highly unpalatable product. In addition, the product had a relatively short useful life, usually limited to less than two weeks, which

often created problems in distribution and handling. Moreover, the processing of such fermented milk as compared with ordinary milk was extremely expensive.

5           The beneficial effects of B. bifidum have also been recently reported (The Milk Industry, September, 1973). Here, it has been established that B. bifidum forms a substantial proportion of the intestinal flora of babies and performs an inhibiting effect on pathogenic  
10 bacteria and the growth of anaerobic putrefactive microorganisms in the intestinal tract.

          Various processes that have been developed to prepare a multi-cultured milk product of this type have  
15 involved using yeast or alcohol which must necessarily interfere with the antibiotic effect of the bacteria since alcohol destroys bacteria. Various yogurt-type products, as distinguished from ordinary dairy yogurt, have been produced by either a mixing together of separate fermented  
20 products such as in United States Patent No. 1,889,817 (Matt) issuing on February 28, 1933; high heat treatment as in United States Patent No. 2,119,599 (Nordsiek) issuing on June 7, 1938; or alcoholic fermentation as in United States Patent No. 2,842,804 (Mishima) issuing on  
25 February 25, 1958. None of these processes, however, produces a palatable product, and attempts to improve the flavor have not been satisfactory.

          The addition of flavors and sugar, particularly  
30 fresh fruits and berries, to the mixes undergoing the incubation stage has met with disfavor in that the incubation conditions have caused loss of flavor and color in the flavoring additives. Moreover, because the digestion of fruits and milk involve different enzymes and  
35 different digestive processes, such mixing may involve



enzymatic and other conflicts ruining any such mixture as a health food. Other processing attempts have resulted in poorly controlled growth conditions which yield inferior quality taste, poor shelf life, as well as substantially  
5 altering the nutritional value of the milk product.

It is the purpose of this invention to provide a new nutritional and therapeutic milk product made by a multi-culturing process that will significantly reduce the  
10 unspent lactose after a yogurt-type fermentation is completed and produce a product that is tasteful and can be digested by persons having an intolerance for lactose, besides being more easily digested by anyone.

15 Another purpose of this invention is to provide a food product with better flavor, consistency, and palatability than previously obtained when culturing a milk product with L. acidophilus or B. bifidum microorganisms.

20 In addition, the ingredients used in this invention not only produce a more desirable milk food product but also provide for an inexpensive process that transforms the percentage of lactose into glucose and  
25 galactose by an amount heretofore thought impossible, thereby providing a food product that is more easily digestible by anyone and particularly by the multitude of people who cannot drink milk.

30 A basic purpose of this invention is to break down as much lactose in milk as possible into glucose and galactose, while maximizing the amount of the enzyme lactase in the finished product through culturing, beyond any procedure presently known.

35

Another basic purpose of this invention is to provide a food product, pleasing to the taste, to act as a vehicle for the ingestion of L. acidophilus and B. bifidum.

5. This invention depends upon the finding that commercial cultured yogurt ordinarily considered to be fully cultured may be cultured further through the use of Bifidobacterium bifidum, and/or other Lactobacilli and that yogurt considered to be fully cultured by L.  
10 bulgaricus and S. thermophilus at about 40°C to about 46°C may be cultured still further by the addition of Bifidobacterium bifidum and/or other Lactobacilli to reduce pH while breaking down lactose and increasing  
15 lactase. The foregoing represents a radical departure from the ordinary art of making cultured dairy products.

An advantage of re-culturing beyond usual culturing is the result of an additional breakdown of lactose into monosaccharides, thus making the product  
20 still more digestible for those individuals allergic to milk.

A further advantage of this invention is that multi-culturing with Bifidobacterium bifidum introduces  
25 more anaerobic bacteria which may survive in the digestive tract, a quality considered desirable by many doctors.

A fundamental function of this invention is to culture what is considered a fully cultured food product  
30 still further into a multi-cultured food product. The multi-culturing process, as described in this patent, breaks down lactose in milk (or milk sugar) into glucose and galactose, while enhancing the amount of the enzyme lactase in the finished product. Lactase is an enzyme  
35 found in limited quantity in raw milk, but is destroyed by

pasteurization, and thus conceivably produces a dietary shortage of lactase. The multi-culturing process of this invention, more fully than by any other method yet offered, overcomes this lactase shortage and yet maintains  
5 the flavor advantages of yogurt, or of cottage cheese.

Other objects of the invention will be apparent from the following discussion and description of this invention.

10

Multi-cultured buttermilk or yogurt may also be used in the preparation of multi-cultured cottage cheese in a manner described herein.

15

The general method of the present invention is comprised of numerous sequential steps. Initially, a first and a second culture are produced separately. The  
20 first culture is comprised of Lactobacillus bulgaricus or Streptococcus thermophilus, which is the standard culture used in the manufacturing of yogurt. The second culture is comprised of Bifidobacterium Bifidum or Lactobacillus acidophilus or any other fermentive substance such as a  
25 lactic acid producing microorganism. By stating the composition of the first and second cultures as an alternative combination it is intended to include those compositions comprised of a single microorganism or the microorganism pair. These are intended merely as  
30 alternative embodiments. In the preferred embodiment, however, the first culture is comprised of equal cell counts of L. bulgaricus and S. thermophilus. As is common in the art, the cultures would be produced in advance of the actual time it is intended to make the multi-cultured  
35 milk product.

In the first step of the actual process, milk or milk products such as cream, partially skimmed milk and skim milk are combined in a mixing tank with concentrated skim milk, non-fat dry milk or other milk derived ingredients used to standardize milk-solids-not-fat.

In the second step the combined ingredients in the mixing tank are mixed thoroughly. The mixing step can be accomplished mechanically or in conjunction with the pasteurization step that follows. Pasteurization may be accomplished in any of the methods well known in the art. In one embodiment of the invention, however, it should be noted that the pasteurization step can be entirely avoided, as is subsequently explained, because the successive fermentation steps achieve sufficient destruction of the harmful entities usually destroyed in the pasteurization process to render a very healthful product.

After the mixing and pasteurization steps are completed, if pasteurization is desired the entire mixture is cooled to a first fermentation temperature in the range of about 40°C to about 50°C. After the mixture reaches the desired temperature it is inoculated with the first culture initiating a first fermentation. The first fermentation is allowed to continue at the first fermentation temperature for a time sufficient enough that an amount of lactic acid is generated to produce a first cultured product.

The first cultured product is then cooled to a second fermentation temperature in the range of about 35°C to about 39°C. When the first cultured product is sufficiently cooled and thus arresting any further activity of the first culture the first cultured product

is inoculated with the second culture to initiate a second fermentation and produce a second cultured product. The second fermentation is allowed to continue at the second fermentation temperature for a time  
5 sufficient enough to produce a palatable and healthful multi-cultured food product.

Yogurt is generally made from a mix standardized from whole, partially defatted milk, condensed skim milk,  
10 cream and nonfat dry milk. Alternatively, milk may be partly concentrated by removal of about 15% to about 20% water in a vacuum pan. Supplementation of milk-solids-not-fat (MSNF) with nonfat dry milk is the preferred industrial procedure. All dairy raw materials should be  
15 of a high bacteriological quality, ingredients containing mastitis milk and rancid milk should be avoided. Milk partially fermented with contaminant organisms, or milk containing antibiotic and sanitizing chemical residues cannot be used for yogurt production as it interferes with  
20 the activity of the beneficial microorganisms.

The milk fat levels in yogurt range from about 1.00% to about 3.25% The proposed federal standards of identity (Federal Register 1977) define the product in  
25 three categories. The product containing a minimum of 3.25% milk fat is called yogurt. Low-fat yogurt contains not less than 0.5% and not more than 2% milk fat. The product containing less than 0.5% milk fat is labeled as nonfat yogurt. In all the categories of yogurt, a MSNF  
30 minimum of 8.25% and a titratable acidity minimum of 0.5% lactic acid is required.

The ingredients are: cream, milk, partially skimmed milk and skim milk, alone or in combination.  
35 Concentrated skim milk, non-fat dry milk or other milk

derived ingredients may be used to standardize MSNF content of the mixture. Presumably, the milk derived ingredients include casein, sodium and calcium caseinates, whey protein concentrates alone or in combination. The  
5 use of milk derived ingredients is permitted on the condition that the ratio of protein to total nonfat solids of the food and the protein efficiency ratio of all protein present should not be diminished. Additives permitted are nutritive carbohydrate sweeteners, coloring,  
10 stabilizers and fruit preparations for flavoring yogurt. The culture is specified as Lactobacillus bulgaricus and Streptococcus thermophilis, plus Bifidobacterium bifidum, Lactobacillus acidophilus, or any other Lactobacilli, Lactobacillus or Bifidobacterium used in further culturing.

15

Nutritive carbohydrates used in the yogurt making process are similar to the sweeteners used in ice cream and other frozen desserts described by Arbuckle (1972). Sucrose is the major sweetener used in yogurt production:  
20 Sometimes, corn sweeteners and honey may also be used. The level of sucrose in the yogurt mixture appears to affect the production of lactic acid and flavor by the yogurt culture. Bills et al., (1972) reported a decrease in acetaldehyde production at 8% or higher concentration  
25 of sucrose. Sucrose may be added in a dry, granulated, free-flowing, crystalline form or as a liquid sugar containing 67% sucrose. Liquid sugar is preferred for its handling convenience in large operations. However, storage capability in sugar tanks along with heaters,  
30 pumps, strainers and meters is required. The corn sweeteners, primarily dextrose, usually enter yogurt via the processed fruit flavorant in which they are extensively used for enhancing flavor. Corn syrup solids up to a 6% level are usually used in frozen yogurt.  
35 Non-nutritive sweeteners (e.g., Ca saccharin) have been

used along with maltol to produce a product containing about 50% fewer calories than normal sweetened yogurt.

Lactase has been suggested for hydrolysis of  
5 lactose to a sweeter mixture of glucose and galactose in yogurt, thereby reducing the level of sucrose required to achieve a constant degree of sweetness (Kosikowski and Wierzbicki 1971, Engel 1973). Goodenough and Kleyn (1976) investigated qualitative and quantitative changes in  
10 yogurt during its manufacture. They reported average lactose concentration of 8.5% in yogurt mix. Upon fermentation, the lactose level dropped to 5.75% with a concomitant increase of 1.20% galactose. Glucose was detected only in trace quantities. Commercial yogurts  
15 had, on an average, above 4.0 pH, 4.06% lactose, 1.85% galactose and 0.05% glucose, but multi-cultured yogurt may have a still lower pH.

The primary purpose of using a stabilizer in  
20 yogurt is to produce smooth body and texture, impart gel structure and reduce wheying off or syneresis. The stabilizer increases shelf life and provides a reasonable degree of uniformity to the product. Stabilizers function by forming gel structures in water, thereby leaving less  
25 free water for syneresis. In addition, some stabilizers complex with casein or milk. A good yogurt stabilizer should not impart any flavor, be effective at low pH values, and should be easily dispersible at the normal working temperatures in a dairy plant. The stabilizers  
30 generally used in yogurt are gelatin, vegetable gums like carboxymethyl cellulose, locust bean, guar and seaweed gums like alginates and carrageenans (Hall 1975).

Gelatin is derived by irreversible hydrolysis of  
35 proteins collagen and ossein; it is used in a level of

0.3% to about 0.5% to get a smooth shiny appearance in refrigerated yogurt. Gelatin is a good stabilizer for frozen yogurt. The term Bloom refers to the gel strength as determined by a Bloom gelometer under standard

5. conditions of the test. Gelatin of Bloom strength 225 or 250 is commonly used. Gelatin level should be directed to the consistency standards of yogurt. Levels above 0.35% tend to impart to yogurt of relatively high milk solids a curdly appearance upon stirring. At temperatures below

10 10°C, the yogurt acquires a pudding-like consistency. Gelatin tends to degrade at ultra-high temperatures of processing thus its activity is temperature dependant. Yogurt gel is considerably weakened by a rise in

15 temperature. Furthermore, being an animal product of unknown origin, gelatin is generally not acceptable in Kosher yogurts. The seaweed gums impart a desirable viscosity as well as gel structure to yogurt. Algin and sodium alginate are derived from giant sea kelp. Carrageenan is made from Irish moss and compares favorably

20 with 250 Bloom gelatin in stabilizing value. These stabilizers are heat stable and promote stabilization of yogurt gel by complex formation with  $\text{Ca}^{++}$  and casein. Among the seed gums, locust bean gum or carob gum is derived from the seeds of a leguminous tree. Carob gum is

25 quite effective at low pH levels. Guar gum is also obtained from seeds and is a good stabilizer for yogurt. It is readily soluble in cold water and is not affected by high temperatures used in the pasteurization of the yogurt mixture. Carboxymethyl cellulose is a cellulose product

30 and is effective at high processing temperatures.

The stabilizer system used in yogurt mix preparation is generally a combination of various vegetable stabilizers to which gelatin may or may not be

35 added. Their ratios as well as the final concentration



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(generally in the range of 0.5% to about 0.7%) in the product are carefully controlled to get desirable effects. Other stabilizers reportedly used are agar and pectin (Humphreys and Plunkett 1969).  $\text{CaCl}_2$  may be  
5 useful in controlling whey separation (Pette and Lolkema 1951). For a detailed description of various industrial gums, see Whistler (1973).

The fruit preparations for blending in yogurt are  
10 specially designed to meet the marketing requirements for the type of yogurt. They are generally present at levels of about 15% to about 20% in the final product (Craven 1975). A majority of the fruits contain natural WONF flavors. There are many types of yogurts marketed in the  
15 United States, such as but not limited to, Fruit-on-Bottom or Eastern Sundae Style, Western Sundae Style and Swiss Style.

In Fruit-on-Bottom or Eastern Sundae Style  
20 Yogurt, 2 oz. of fruit preserves are layered at the bottom followed by 6 oz. of inoculated yogurt mix on the top. No flavorant or sweetener is added to the yogurt. After placing the lids on the cups, incubation and fermentation takes place in the cups. When a desirable pH level in the  
25 range of 4.4 to 4.2 is attained in ordinary yogurt (the yogurt of the present invention would be less), the cups are placed in refrigerated rooms for rapid cooling. For consumption, the fruit and yogurt layers are mixed by the consumer. Fruit preserves have a standard of identity. A  
30 preserve is made from 55% sugar, and a minimum of 45% fruit by cooking until the final soluble solids content is 68% or higher (65% in the case of certain fruits) (Gross 1974). Frozen fruits and juices are the usual raw material. Commercial pectin, 150 grade, is normally  
35 utilized at a level of 0.5% in preserves and the pH is

adjusted to a range of 3.0 to about 3.5 with a food grade acid, such as citric acid, during manufacture of the preserves.

5           In Western Sundae Style Yogurt fruit preserves or special fruit preparations may form the bottom layer. The top layer comprises yogurt containing sweetener, flavorant and food color representing the fruit on the bottom. The flavorant level is usually in the range of about 2% to  
10 about 4% in the top layer. In other respects, this yogurt is identical to Eastern Sundae Style.

          In Swiss Style Yogurt, also known as Continental Style, French Style and Stirred yogurt, the fruit  
15 preparation is thoroughly blended in the yogurt after culturing. Stabilizers are necessary in this form of yogurt unless MSNF levels are relatively high (in the range of about 14% to about 16%). In this style yogurt, cups are filled with the blended yogurt mixture and  
20 fruit. Upon refrigerated storage for 48 hours, the clot is reformed to exhibit a fine body and texture. Overstabilized yogurt possesses a solid-like consistency and lacks a refreshing character. Yogurt should not be so thin that it is drinkable; it should melt in the mouth  
25 without chewing.

          Flavors and certified food colors are usually added for eye appeal and better flavor profile. The fruit base should meet the following requirements: a) exhibit  
30 true color and flavor of the fruit when blended with yogurt, b) be easily dispersible in yogurt without causing texture defects, phase separation or syneresis (in this regard the pH of the fruit base should be compatible with the yogurt pH); and, c) have a microbiological quality so  
35 that yeasts and molds can be controlled in the final product to prevent spoilage and to extend shelf life.

Fruit preserves do not necessarily meet all these requirements, especially flavor, sugar level, consistency and pH. Accordingly, special fruit bases having the following composition are designed for use in stirred  
5 yogurt.

	Fruit .....	about 17% to about 41%
	Sugar .....	about 22% to about 40%
	Corn syrup solids .....	about 10% to about 24%
10	Modified food starch .....	about 3.5% to about 5.0%
	Fruit flavor, artificial .....	about 0.1%
	Fruit flavor, natural WOLF .....	about 1.25%
	Color .....	about 0.01% or to specifications
15	Potassium sorbate .....	about 0.1%
	Citric acid added to get pH in the range of about 3.7 to about 4.2	

$\text{CaCl}_2$  and certain food grade phosphates are  
20 also used in several fruit preparations. The soluble solids range from about 60% to about 65% and viscosity is standard to  $5 \pm 1.5$  Bostwick units (cm), 30 second reading at 24°C. Standard plate counts on the fruit bases are generally less than 55/g. Coliform count, yeast and mold  
25 count is less than 10/g. The fruit flavors vary in popularity in different parts of the country and during different times of the year. In general, more popular fruits are: strawberry, raspberry, blueberry, peach, cherry, orange, lemon, purple plum, boysenberry, spiced  
30 apple, apricot and pineapple. Blends of these fruits are also popular. Fruits used in yogurt base manufacture may be frozen, canned, dried or combinations thereof. Among the frozen fruits are: strawberry, raspberry, blueberry, apple, peach, orange, lemon, cherry, purple plum,  
35 blackberry and cranberry. Canned fruits are: pineapple,

peach, mandarin orange, lemon, purple plum and maraschino cherry. The dried fruit category includes apricot, apple, and prune. Fruit juices and syrups are also incorporated in the bases. Sugar in the fruit base functions in  
5 protecting fruit flavor against loss volatilization and oxidation. It also balances the fruit and the yogurt flavor. The pH control of the base is important for fruit color retention. The color of yogurt should represent the fruit color in intensity, hue and shade. The base should  
10 be stored under refrigeration to retain optimum flavor and to extend shelf life.

The yogurt starter or first culture is a critical ingredient in yogurt manufacture with important  
15 consequences for texture and flavor. For the practical aspects of yogurt culture, see Tramer (1973) and David (1975). Freedom from contaminants, vigorous growth in the yogurt mixture, good flavor, body and texture production and a reasonable resistance to phages and antibiotics are  
20 primary requirements of yogurt starter. Equal cell numbers of Lactobacillus bulgaricus and Streptococcus thermophilus are desirable for flavor and texture production. The lactobacilli grow first liberating amino acids, glycine and histidine, stimulating the growth of  
25 streptococci (Bautista et al., 1966). Tramer (1973) demonstrated differences in the comparative acid production ability of various strains of yogurt starters in commercially autoclaved versus heat-treated (95°C for 30 minutes) milk. The rate of acid production by the  
30 yogurt starter should be synchronized with plant production schedules. Using frozen yogurt starter concentrates, incubation period of 5 hours at 45°C, 11 hours at 32°C and from 14 to about 16 hours at 29°C to about 30°C is required for yogurt acid development. Using  
35 bulk yogurt starters at a 1% inoculum level (that is, 1

part yogurt starter for every 100 parts of the mixture to which it is added) the period is about 2.5 to about 3.0 hours at about 45°C, 8 to about 10 hours at 32°C and 14 to about 16 hours at 29°C-30°C (Yeager 1973). Stone et al. 5 (1975) reported that milk, UHT pasteurized at temperatures from about 115.6°C to about 157.2°C and a holding time of 0.02 seconds, exhibited higher starter activity in comparison with vat pasteurized milk.

10 The production of flavor by the yogurt starter is a function of time as well as sugar content of the yogurt mixture. Gorner et al. (1968) reported that acetaldehyde production in yogurt takes place predominantly in the first 1 or 2 hours of incubation; eventually, 23 to about 15 55 ppm of acetaldehyde is found in yogurt. Hamdan et al. (1971) reported acetaldehyde levels of 22 to about 26 ppm in their cultures at the fifth hour of incubation, which declined in later stages of incubation. Yogurt flavor is typically ascribed to the formation of lactic acid, 20 acetaldehyde, acetic acid and diacetyl.

The milk coagulum during yogurt production results from an increase in acidity due to the activity of the yogurt starter. The streptococci are responsible for 25 increasing the acidity of yogurt mix to the range of about pH 5.5 to about pH 5.0 and the lactobacilli are primarily responsible for further increasing of acidity to pH 4.4 approximately. Attempts have been made to improve the viscosity and to prevent syneresis of yogurt by including 30 a slime producing strain of Streptococcus filant or Streptococcus bactis variety hollandicus (Galislout and Hassing 1968; Busch-Johannsen et al. 1971; Tramer 1973). The texture of yogurt tends to be coarse or grainy if it is allowed to develop firmness prior to stirring or if it 35 is disturbed at acidity values higher than pH 4.6. Rennet

addition to the yogurt mixture, excessive whey solids use and incomplete blending of the mixture's ingredients are additional causes of a coarse texture. Homogenization treatment and fat content tend to favor smooth texture.

5 Gassiness in yogurt may be attributed to defects in yogurt starters or contamination with spore forming *Bacillus* species, coliforms or yeasts, producing excessive CO<sub>2</sub> and hydrogen. In comparison with plate-type heat exchangers, cooling with tube-type heat exchangers cause  
10 less damage to yogurt structure (Mana 1973). Further, loss of viscosity of yogurt may be minimized by well designed booster pumps, metering units and valves involved in yogurt packaging.

15 The acidity of ordinary yogurt during storage continues to increase from about pH 4.62 to about pH 4.15 in about 6 days of storage at 4°C (Gavin 1965). High storage temperatures accelerate the increase in acidity.

20 Most yogurt manufacturers incorporate from 5% to about 7% sucrose in their yogurt mixture prior to culturing. Tramer (1973) reported that various strains of yogurt cultures responded differently to various levels of sucrose. No inhibition of culture activity was noticed up  
25 to 5.5% sucrose concentrations. At higher sucrose levels, acid production by the yogurt starter was partially inhibited. This effect was primarily ascribed to the stress on lactobacilli and was related to the total solids level in yogurt. Total solids, consisting of milk solids  
30 and sweeteners, above a level of 22% inhibit Lactobacillus bulgaricus.

The inhibition of yogurt starter is also caused by antibiotic residues in milk. Mocquot and Hurel (1970)  
35 reported that both Streptococcus thermophilus and

Lactobacillus bulgaricus are affected by 0.005 IU/ml of penicillin, 0.066 IU/ml of aureomycin and 0.38 IU/ml of streptomycin. Streptococcus thermophilus is exceedingly sensitive to penicillin. It is affected at 0.01 IU/ml and 5 yogurt production ceases at 0.03 IU/ml.

Phages are not a practical threat to yogurt making if frozen yogurt starters are used and proper rotation is practiced along with high sanitation standards 10 in the plant. Phages for yogurt cultures, however, have been isolated (Reinhold and Reddy 1973; Kosikowski 1977). Hypochlorites and quarternary ammonium compounds also inhibit yogurt cultures (Bouchez and van Bellegham 1971).

15           Pasteurization or heat treatment of the raw milk at 85°C for 30 minutes, or its equivalent, is an important step in the manufacture of yogurt so that the product is sterilized and any undesirable bacteria are eliminated. The heat treatment a) produces a sterile medium for the 20 exclusive growth of the yogurt starter; b) removes air from the medium to produce a more conducive medium for microaerophilic lactic cultures to grow; c) effects thermal breakdown of milk constituents, especially proteins, releasing peptones, sulfhydryl groups which 25 provide nutrition and anaerobic effects for the yogurt starter; and, d) denatures and coagulates milk albumins and globulins which enhance the viscosity and produce custard-like consistency in the product.

30           It should be noted, however, that according to one embodiment of my invention, the heat treatment step is not employed. Instead of pasteurizing the milk, the action of the various microbes is used to destroy those microorgasms harmful to mankind. Homogenization also aids 35 in the texture development and, additionally, it

alleviates the surface creaming and wheying off problem. Ionic salt balance in milk is also involved in the wheying off problem.

5 Plain yogurt normally contains no added sugar or flavors to offer the consumer natural yogurt flavor for consumption as such or an option of flavoring with other food materials of the consumer's choice. In addition, it may be used for cooking or for salad preparation with  
10 fresh fruits preferred by the consumer. Also, the size and type of the package may be geared to the market demand, however, wax coated cups as well as plastic cups and lids are the chief packaging materials used in the industry.

15

Multi-cultured yogurt may be produced by adding one additional step to the normal yogurt-making process; that is adding a minimum of 1% (that is, 1 part culture for every 100 parts yogurt) Bifidobacterium bifidum, such  
20 as Eugalen Forte supplied by Bio-Nutritional Products of Harrison, New York, up to approximately 5% (5 parts culture for every 100 parts yogurt), to the yogurt product previously cultured to increase the acidity by an additional pH .5 approximately. It should be noted that  
25 lower pH increases shelf life by a factor of two. The time required shall be determined by acidity level and shall be suitably sufficient to produce a palatable and healthful product.

30

L. acidophilus may be used in combination with Bifidobacterium bifidum. Using L. acidophilus singly, however, simply modifies the flavor to a sharper taste. Additional culturing with Bifidobacterium bifidum alone produces a sweeter flavor than L. acidophilus or L.  
35 acidophilus in combination with Bifidobacterium bifidum, and also adds protection against yeast formation.



In addition, Bifidobacterium bifidum is an anaerobic bacteria with specialized qualities discovered by Japanese scientists. Interactions between Bifidobacterium bifidum N 4 and Escherichia Coli K-12 in their Mixed Cultures by 5 Euchi Hara, Koihei Yazawa, Hiroshi Nakamura, and Zenzo Tamura, Faculty of Pharmaceutical Sciences, University of Tokyo, August 21, 1978, summarizes:

10 "The interactions between Bifidobacterium bifidum N4 (B. bifidum) and Escherichia K-12 (E. coli) were investigated in their mixed cultures.

15 Under conditions in which both bacteria grew well in their pure culture, B. bifidum inhibited the growth of E. coli even when the latter was inoculated at  $10^4$  fold and preincubated for 41 hours. The inhibition in the mixed cultures 20 appeared when the pH values were reduced below 4.6..."

Selective Localization and Growth of Bifidobacterium bifidum in Mouse Tumors Following Intravenous Administration, by Noritaka T. Kimura, 25 Shun'ichiro Taniguchi, Ken Aoki, and Tsuneo Baba, Kyushu University, Japan, June, 1980, offers the following:

30 "A strain of domestic bacteria, Bifidobacterium bifidum (Lac B), which is nonpathogenic and anaerobic, selectively localized and proliferated in several types of mouse tumors following i.v. administration. None of the same bacilli 35 could be detected in the tissues of

healthy organs such as the liver, spleen,  
kidney, lung, blood, bone marrow, and  
muscle 48 or 96 hrs. after i.v.  
administration into tumor-bearing mice."

5

Bifidobacterium bifidum, moreover, constitutes  
99% of the intestinal flora of the healthy nursing infant,  
according to Role of Dietary Lactobacilli in  
Gastrointestinal Microecology, by Khem M. Shahani and  
10 Amadu D. Ayebo, Department of Food Science and Technology,  
University of Nebraska, published as paper No. 6050,  
Journal Series, Nebraska Agricultural Experiment Station.  
The reported research was conducted under project No.  
16-026, supported in part by a grant from the National  
15 Dairy Council.

A multi-cultured food product made with  
Bifidobacterium bifidum for a secondary culturing is  
recommended because of the certainty that such bacteria,  
20 cultured at 37°C, normal human body temperature, will  
dominate bacteria cultivated at 21°C. The use of  
multi-cultured yogurt containing Bifidobacterium bifidum,  
however, should adequately culture the plain yogurt  
because Bifidobacterium bifidum cultures at 37°C whereas  
25 plain yogurt bacteria culture at about 40.5°C to about  
45.5°C in normal usage. The bacteria used in culturing  
plain yogurt will not implant in the human digestive tract  
because the required temperature is too high.

30

In one embodiment of the present invention whole  
milk was fermented with a 1% Bifidobacterium bifidum  
culture at 37°C for 48 hours. This embodiment produces  
some unusual results. Culturing with B. Bifidum yields a  
dairy product similar to buttermilk in consistency but  
35 having a very sweet taste with a curd much smaller than

expected. Two containers of whole milk, one fresh and the other stale, were used. After 24 hours the bacteria in the stale ingredients overwhelmed the B. bifidum bacteria and reduced the sweet taste.

5

The 48 hour fermentation time may be a disadvantage in a commercial dairy plant. single culturing with B. bifidum will also work with dairy ingredients other than whole milk and therefore this embodiment is not so limited. It is also within the scope of this invention to make a B. bifidum yogurt by substituting B. bifidum for L. bulgaricus and/or S. thermophilus and/or any other suitable bacteria or bacterium.

15

Bifidobacterium bifidum, L. acidophilus and yogurt bacteria all produce lactase in the process of souring. Additional souring beyond the norm for a cultured product does not simply increase lactase; it does more. As described hereinabove, the additional fermentation step improves flavor in buttermilk, yogurt, solid spread or cottage cheese, as usually produced. The further culturing of commercial buttermilk with Bifidobacterium bifidum is considered unique. The yogurt may be turned into a spread by increasing the amount of solids to something like 50%. Otherwise, the multi-cultured yogurt is the same as described.

25

This invention also relates to cottage cheese made by substituting a double-cultured dairy product comprised of B. bifidum or L. acidophilus having the consistency of buttermilk to cottage cheese curd during the creaming operation, rather than some form of sweet skimmed milk, sweet cream, or sweet milk, either condensed or not condensed.

35

As stated, in processing, the pH should be reduced by .5 approximately. However, the pH reduction in plain yogurt by any significant amount by re-culturing represents the breaking down of more lactose, a major factor in this invention.

Wilcox (1971) reviewed the processes for making yogurt with polyunsaturated corn oil, instant yogurt and yogurt enriched with vitamin C.

10

Soft serve yogurt, hard packed yogurt and novelty items based upon yogurt are relatively new products getting an enthusiastic response by the consumer. Push-ups, frozen yogurt on a stick, skippy cups and tetrapaks are being manufactured and marketed.

The frozen yogurt base mix may be manufactured in a cultured dairy plant and shipped to a soft serve operator or an ice cream plant. Alternatively, the mix may be prepared and frozen in an ice cream plant. The following formulation is generally used: milk fat in the range of about 1.5% to about 2.0%, MSNF in the range of about 13% to about 15%, 250 Bloom gelatin in the range of about 0.15% to about 0.20%, sucrose in the range of about 7% to about 10% and corn syrup solids (24-26 DE) in the range of about 4% to about 5%. These ingredients (except one half the sugar) are standardized in a blend tank and pasteurized at 88°C for 40 minutes. The mixture is then homogenized at a temperature in the range of about 58°C to about 63°C at 1500 psi, then cooled to 44°C. Yogurt culture is then inoculated and incubation of the mixture is continued until pH 3.9 is attained. The yogurt mixture is then cooled to 25°C and the remaining sugar and fruit are then blended. Special fruit preparations designed for frozen yogurt are used at a level of about 15% to about

20%. This mixture is then frozen in an ice cream freezer at about 50% to about 60% overrun, packed and hardened similar to ice cream. To obtain a soft serve product, a soft serve freezer is used at a draw temperature of -8°C.

5

A quality control program for yogurt manufacture includes the control of product flavor, body, texture, color, process and composition. The flavor defects are generally described as too intense, too weak or  
10 unnatural. The sweetness level may be excessive, weak or may exhibit corn syrup flavor. The flavor may be too tart, weak or atypical. The ingredients used may impart undesirable flavors like stale, metallic, old ingredients, oxidized, rancid or unclean. Lack of control in  
15 processing procedures may cause overcooked, caramelized or too tart flavor. Proper control of processing parameters and ingredient quality insures good flavor.

In hard pack frozen yogurt, a coarse and icy  
20 mixture may be caused by storage temperature fluctuations. Sandiness may be due to lactose crystals resulting from too high levels of milk solids or whey solids. A soggy or gummy defect is caused by too high a MSNF level or a too high sugar content. A weak body  
25 results from too high an overrun and insufficient total solids.

Color defects may be caused by the lack of intensity or authenticity of hue and shade. Proper  
30 blending of fruit purees and yogurt mixture is necessary for uniformity of color. The compositional control tests are: fat, moisture, pH, and overrun (for frozen yogurt) and microscopic examination of yogurt starter to insure a ratio of 1:1 in Lactobacillus bulgaricus and Streptococcus  
35 thermophilus. Good microbiological quality of all

ingredients is necessary. As a guideline, raw milk and cream should contain less than 500,000 and 800,000 bacteria/ml, respectively. Pasteurized fluid dairy products should not exceed 50,000 counts/g or ml.

- 5 Coliform counts for pasteurized products should not exceed 10/g or ml. Also periodic checks for yeast and mold counts on fruit preparations would be useful.

Although the invention is described in terms of  
10 particular ingredients and in what is conceived to be the most practical and preferred method, it is recognized that departures made that fall within the scope of this invention, which is not to be limited to the details disclosed herein but is to be accorded the full scope of  
15 the claims so as to embrace any and all equivalent ingredients and methods, including other starters used in cultured products, such as S. lactis, S. cremoris, L. citrovorum, S. diacelelactics and S. durans to achieve maximum reduction of pH which improves keeping qualities  
20 because many bacteria cannot survive a low pH.

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CLAIMS:

1. A method of producing a multi-cultured food product which comprises:
  - 5 a. producing separately a first culture wherein said culture comprises Lactobacillus bulgaricus or Streptococcus thermophilus;
  - b. producing separately a second culture wherein said culture comprises any fermentive substance;
  - 10 c. mixing in a mixing tank cream, milk, partially skimmed milk and skim milk, alone or in combination, where concentrated skim milk, non-fat dry milk or other milk derived ingredients are used to standardize  
15 milk-solids-not-fat;
  - d. pasteurizing the mixture of step (c) where the mixture is cooled only to a fermentation temperature in the range of about 40°C to about 50°C to produce a prepared mixture;
  - 20 e. inoculating the prepared mixture with the first culture to produce a prepared milk fluid;
  - f. fermenting the prepared milk at a temperature in the range of about 40°C to about 50°C for  
25 a time sufficient enough that an amount of lactic acid is generated to produce a first cultured product;
  - g. cooling the first cultured product to a temperature in the range of about 35°C to  
30 about 39°C;
  - h. inoculating the cooled first cultured product with the second culture to produce a second cultured product; and
  - 35 i. fermenting the second cultured product for a time sufficient to produce a palatable and healthful multi-cultured food product.

2. The method of claim 1 wherein the second culture is Bifidobacterium bifidum.

3. The method of claim 1 wherein the second culture is Lactobacillus acidophilus.

5 4. The method of claim 1 wherein the second culture is comprised of Bifidobacterium bifidum or Lactobacillus acidophilus.

5. The method of claim 1 wherein the second culture is Bifidobacterium bifidum and any other  
10 microorganism.

6. The method of claim 1 wherein the second culture is Lactobacillus acidophilus and any other micro-organism.

7. The method of any of claims 1, 2, 3, 4, 5 or  
15 6 wherein the first culture comprises equal cell counts of Lactobacillus bulgaricus and Streptococcus thermophilus.

8. The method of claim 7 further comprising fermenting the second cultured product for a time sufficient to reduce the second cultured product pH by  
20 about 0.5.

9. The method of claim 7 further comprising adding fruit preparation.

10. The method of claim 9 further comprising blending thoroughly the fruit preparation in the food  
25 product after the second culturing.

11. The method of claim 1 wherein the ingredients of step (c) are derived from certified milk and step (d) is eliminated to the extent that the mixture does not undergo a pasteurization heating.

30 12. The method of claim 7 further comprising adding milk solids to the mixture of step (c) so that said mixture has a consistency between sour cream and butter.

13. The method of claims 1 and 5 wherein the fermentation of the second cultured product takes place at  
35 a temperature in the range of about 36°C to about 38°C.



14. A method of producing a frozen multi-cultured food product which comprises:

- a. preparing a preliminary first culture wherein said culture comprises Lactobacillus bulgaricus or Streptococcus thermophilus;
- b. preparing separately a second culture wherein said culture comprises any fermentive substance;
- c. mixing milk fat, milk-solids-not-fat, gelatin, sucrose and corn syrup solids to produce a mixture;
- d. blending the mixture;
- e. pasteurizing the blended mixture;
- f. cooling the pasteurized blended mixture to a temperature in the range of about 58°C to about 63°C;
- g. homogenizing the mixture of step (f) to produce a precursor;
- h. cooling the precursor to a temperature in the range of about 40°C to about 45°C;
- i. inoculating the cooled precursor with the first culture to produce a product;
- j. fermenting the first product for a time sufficient enough that an amount of lactic acid is generated to produce a preliminary first cultured product;
- k. cooling the first cultured product to a temperature in the range of about 35° to about 39°C.
- l. inoculating the cooled first cultured product with the second culture to produce a second cultured product;
- m. fermenting the second cultured product for a time sufficient enough that the pH is reduced by about 0.5 to produce a

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multi-cultured food product; and

- n. freezing the multi-cultured food product to a suitable hardness to produce a frozen multi-cultured food product.

5 15. A double-cultured dairy product comprised of Bifidobacterium bifidum or Lactobacillus acidophilus.

16. A method of producing a multi-cultured cottage cheese which comprises:

- 10 a. producing a starter culture wherein said culture comprises Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetylactis or a microorganism of the Leuconostoc species;
- 15 b. heating pasteurized skim milk to about 32°C in a vat;
- c. inoculating the heated pasteurized skim milk with the starter culture and rennet to produce a first cultured product;
- 20 d. fermenting the first cultured product for a time sufficient enough to produce a curd with an optimum pH measurement;
- e. cutting the fermented first cultured product to produce a curd and whey mass;
- 25 f. heating the curd mass and whey to a cooking temperature in the range of 54°C to about 58°C;
- g. cooking the curd mass and whey at the cooking temperature for a time sufficient enough to achieve a desired firmness;
- 30 h. draining of the whey;
- i. washing the curd mass with fresh water to remove all whey;
- j. trenching the curd while draining a final wash; and
- 35

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k. creaming the washed curd with a cream dressing comprised of a double-cultured dairy product having B. bifidum or L. acidophilus to produce cottage cheese.

5 17. A single cultured dairy product having Bifidobacterium bifidum.

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**EUROPEAN PATENT APPLICATION**

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⑤④ **Multi-cultured yogurt, solid spread and cottage cheese.**

⑤⑦ This invention is for a multi-cultured yogurt or dairy spread such as cottage cheese and the process for making them. Yogurt is normally produced by inoculating milk with *L. bulgaricus* and *S. thermophilus* then letting fermentation breakdown the milk lactose. The accomplishment of this invention is the recognition that more lactose can be broken down by adding a second fermentation step to the usual yogurt making process. The result is that one maximizes the breakdown of milk lactose into glucose and galactose while enhancing the amount of enzyme lactase in the finished product. *B. bifidum* or *L. acidophilus* are disclosed as the preferred microorganisms used in the second fermentation steps.

**EP 0 122 104 A3**



European Patent  
Office

# EUROPEAN SEARCH REPORT

0122104

Application number

EP 84 30 2251

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
X,D	<p><u>US - A - 4 034 115</u> (J. ROBERTS)</p> <p>* claim 1; column 3, line 54 - column 5, line 19 *</p> <p>--</p>	1-11, 13,15	<p>A 23 C 9/123 19/032 19/076 9/133</p>
X	<p><u>GB - A - 1 043 194</u> (A. BIRD &amp; SONS)</p> <p>* example 3 *</p> <p>--</p>	1-11, 13,15	
A	<p><u>US - A - 3 506 456</u> (B. FLICK)</p> <p>* column 1, line 25 - column 2, line 50 *</p> <p>--</p>	16	
A	<p>J. DAVIS: "CHEESE", vol. III, 1976 Churchill Livingstone LONDON, GB pages 628-637</p> <p>* pages 633-634, paragraph 1; pages 635-636 *</p> <p>-----</p>	16	<p>TECHNICAL FIELDS SEARCHED (Int. Cl. 3)</p> <p>A 23 C</p>
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 12-09-1984	Examiner DESMEDT
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

- 1) Claims 1-16: Multi-cultured food or dairy product
- 2) Claim 17: Single-cultured dairy product

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims: 1-16

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